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(54) Title: **METHODS AND COMPOSITIONS FOR DETERMINING ENZYMATIC ACTIVITY**

(57) **Abstract:** The present invention comprises crystalline polyketide synthases, isolated non-native polyketide synthases having the structural coordinates of said crystalline polyketide synthases, and nucleic acids encoding such non-native polyketide synthases. Also disclosed are methods of predicting the activity and/or substrate specificity of putative polyketide synthase, methods of identifying potential polyketide synthase substrates, and methods of identifying potential polyketide synthase inhibitors.

INTERNATIONAL SEARCH REPORT

In. International Application No
PCT/US 00/20674

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/29 C12N15/54 C12N9/10 C12Q1/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBL, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JEZ J M ET AL.: "Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis." FASEB JOURNAL. MEETING INFO.: ANNUAL MEETING OF THE AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY ON BIOCHEMISTRY AND MOLECULAR BIOLOGY 99 SAN FRANCISCO, CALIFORNIA, USA MAY 16-20, 1999, vol. 13, no. 7, 23 April 1999 (1999-04-23), page A1392 XP002158823 abstract 355	1,6,7, 12-14, 16,17, 22-39
Y	---	13,15, 18,22, 25, 29-31, 34-36
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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In	International Application No
PCT/US 00/20674	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE PDB 'Online! ID 1BI5, 22 June 1999 (1999-06-22) FERRER J-L ET AL.: "Chalcone synthase from Alfalfa" XP002158822 cited in the application the whole document	1, 6, 7, 12-14, 16, 17, 22-39
Y	JUNGHANS H ET AL.: "Stress responses in alfalfa (<i>Medicago sativa L.</i>). 15. Characterization and expression patterns of members of a subset of the chalcone synthase multigene family" PLANT. MOL. BIOL., vol. 22, no. 2, May 1993 (1993-05), pages 239-253, XP002158812 cited in the application Note: 99.7% aa sequence identity of CHS2 with SEQ ID NO:1 in in 389 aa overlap. abstract figures 1-3	13, 15, 18, 22, 25, 29-31, 34-36
Y	RAIBER S ET AL.: "Molecular and enzymatic characterization of two stilbene synthases from Eastern white pine (<i>Pinus strobus</i>). A single Arg/His difference determines the activity and the pH dependence of the enzymes." FEBS LETT., vol. 361, no. 2-3, 20 March 1995 (1995-03-20), pages 299-302, XP002158813 abstract figure 2	13, 22, 29, 31, 34, 36
Y	HELARIUTTA Y ET AL.: "Chalcone synthase-like genes active during corolla development are differentially expressed and encode enzymes with different catalytic properties in <i>Gerbera hybrida</i> (Asteraceae)" PLANT MOLECULAR BIOLOGY, vol. 28, no. 1, April 1995 (1995-04), pages 47-60, XP002158814 abstract figure 1	13, 15, 22, 29, 31, 34, 36
	---	-/-

INTERNATIONAL SEARCH REPORT

In. International Application No

PCT/US 00/20674

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ECKERMANN S ET AL.: "New pathway to polyketides in plants" NATURE, vol. 396, 26 November 1998 (1998-11-26), pages 387-390, XP002158815 cited in the application abstract page 389, left-hand column, line 50-53 ----	13,15, 22,29, 31,34,36
X	TROPP S ET AL.: "Reaction mechanisms of homodimeric plant polyketide synthases (stilbene and chalcone synthase) - a single active site for the condensing reaction is sufficient for synthesis of stilbenes, chalcones, and 6'-deoxychalcones" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 14, 7 April 1995 (1995-04-07), pages 7922-7928, XP002158816 abstract	1,2,7,8, 12
Y	figures 2,3; tables I,II ----	18,25, 30,31, 35,36
X	PREISIG-MÜLLER R ET AL.: "Plant polyketide synthases leading to stilbenoids have a domain catalyzing malonyl-CoA:CO ₂ exchange, malonyl-CoA decarboxylation, and covalent enzyme modification and a site for chain lengthening" BIOCHEMISTRY, vol. 36, no. 27, 8 July 1997 (1997-07-08), pages 8349-8358, XP002158817 abstract	1,2,7,8, 12
Y	tables 1,4 ----	18,25, 30,31, 35,36
A	HUANG W ET AL.: "Crystal structure of beta-ketoacyl-acyl carrier protein synthase II from E. coli reveals the molecular architecture of condensing enzymes" THE EMBO JOURNAL, vol. 17, no. 5, 2 March 1998 (1998-03-02), pages 1183-1191, XP002158818 abstract page 1183, left-hand column, line 1 -right-hand column, line 2 page 1189, right-hand column, line 1-19 ----	12,16, 28,30, 33,35,38
		-/-

INTERNATIONAL SEARCH REPORT

In	ational Application No
PCT/US 00/20674	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHRÖDER J: "A family of plant-specific polyketide synthases" TRENDS IN PLANT SCIENCE, vol. 2, 1997, pages 373-378, XP000979251 the whole document page 376, right-hand column, line 36,37 ---	1,7
A	SCHRÖDER J ET AL: "Stilbene and chalcone synthases: related enzymes with key functions in plant-specific pathways" ZEITSCHRIFT FUER NATURFORSCHUNG. SECTION C. BIOSCIENCES, vol. 45c, no. 1-2, 1990, pages 1-8, XP000564724 the whole document ---	1,7
P,X	FERRER J-L ET AL.: "Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis" NATURE STRUCTURAL BIOLOGY, vol. 6, no. 8, August 1999 (1999-08), pages 775-784, XP000979629 the whole document ---	1,2,6-8, 12-18, 22-39
P,X	JEZ J M ET AL.: "Dissection of malonyl-coenzyme A decarboxylation from polkyetide formation in the reaction mechanism of a plant polyketide synthase" BIOCHEMISTRY, vol. 39, no. 5, 8 February 2000 (2000-02-08), pages 890-902, XP002158820 the whole document ---	1-14, 17-20, 22-39
T	HE M ET AL.: "Structural modeling and site-directed mutagenesis of the actinorhodin beta-ketoacyl-acyl carrier protein synthase" JOURNAL OF BACTERIOLOGY, vol. 182, no. 9, May 2000 (2000-05), pages 2619-2623, XP002158821 abstract -----	12,16, 28,30, 33,35,38